## REMARKS

Claims 1-19 were pending.
Claims 1-19 are rejected.
Claims 1, 4, 8, 9, 10, 11, 12 and 15 are amended.
Claim 20 is new.
Claims 1-20 are pending.

**Amended Claims and New Claim 20**

Claim 1 has been amended to eliminate component (i) and to add the phrase selected from the group consisting of components (iii), (iv), (v) and (vi).

Thus clearly the fermentation liquor is treated with at least two different components. Component (ii) is essential and must be combined with at least one of (iii), (iv), (v) or (vi).

Claim 1 is further amended to require the additional step of flocculating the suspended solids containing lignin. Basis may be found on page 5, line 9 and page 9, line 15.

Claim 1 additionally has been amended to delete the phrase "is characterized by a treatment system which" and inserting "adding to the fermentation liquor."

Claims 4, 8, 9, 10, 11 and 12 have been amended to delete the reference to component (i) as this has been deleted in claim 1.

Claim 15 has been amended to delete the phrase "application of the treatment system" and replace with the "addition step".
The basis for this amendment may be found on page 9, lines 6-9.

New claim 20 is supported by the disclosure on page 5, line 9.

No new matter has been added.

**35 USC 112, second paragraph**

Claims 1-19 are rejected under 35 USC 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicants have amended claim 1 to eliminate the phrase "characterized by a treatment system.

Claim 15 has been amended to delete the reference to the treatment system as this is no longer present in claim 1.

No new matter has been added.

**35 USC 103(a)**

**Claims 1-3, 13, 17 and 19 are rejected under 35 USC 103(a) as being unpatentable over Brink, US 5,536,325 in view of Minowa.**

Examiner alleges that Brink US 5,536,325 discloses a process for separating suspended solids from a fermentation liquor and that the liquor removed from the distillation stage would comprise water, lignin and BOD.

Claim 1 requires a solid-liquid separation stage which comprising adding to the fermentation liquor component (i) AND at least one component selected from the group consisting of (iii), (iv), (v) and (vi). Furthermore claim 1 require flocculation of the suspended solids containing lignin.

Brink discloses a process wherein the hydrolysate obtained from lignocellulosic material has already been separated from insoluble biomaterial such as lignin, before said hydrolysate is neutralized and separated from contaminants resulted from neutralization and finally subjected to a fermentation stage.

The separation of insoluble biomass including lignin from the liquid hydrolysate in Brink is evident from col. 5, lines 58-62 and fig. 4, where the solids remaining after the second hydrolysis step are separated by centrifugation and subjected to wet oxidation. Likewise, another passage referring to fig. 1 describes that the slurry obtained in the stage II hydrolyser is subjected to several separators, and finally the solids, consisting primarily of lignin may be subjected to wet oxidation (cf. fig. 1, col. 4, lines 25-51).

And also, col. 14, lines 36-38 confirm that the wet oxidation step serves to break down lignin. That is, the fermentation liquor does not comprise lignin, contrary to the fermentation liquor of the present process.

Secondly, the solids-liquid separation stage referring to fig. 4 of Brink using ferric and aluminum salts as flocculants is carried out with a neutralized mixture. For example, col. 9, lines 10-15 teaches that various inorganic substances are added to the system to adjust pH (either to acidfy or to neutralize) or to flocculate and to provide nutrients for fermentation.

Thus the ferric and aluminum salts used in Brink are NOT added to the fermentation liquor as presently defined in claim 1 (the fermentation liquor according to claim 1 is produced in a fermentation process for the production a fermentation product).

Thus there is no suggested by Brink to add such salts to a fermentation liquor.

Lastly, Brink makes no suggestion or teaching to flocculate using the two components as presently claimed.

Minowa et al. describes the use of a cationic and anionic polymeric coagulant in dewatering of a fermentation stillage obtained from buckwheat or rice.

Minowa only discloses anionic or cationic coagulants which are known to be low molecular weight polymers.

Claim 1 requires the presence of
ii.) a cationic polymer having high intrinsic viscosity (IV) of at least 4 dl/g and
a second component selected from the group consisting of components (iii), (iv), (v) and (vi).

Minowa makes no suggestion to add a cationic polymer having a high intrinsic viscosity of at least 4 dl/g but instead suggest only the addition of a cationic coagulant (known to be low molecular weight polymers). As Minowa makes no reference to a high viscosity cationic polymer, and Brink makes no reference at all to any polymeric flocculants, the combination of Brink with Minowa does not arrive at the presently claimed process.

Furthermore, claim 1 requires the additional step of flocculating the suspended solids containing lignin.

Minowa discloses the use of anionic and cationic coagulants for dewatering of fermentation stillage for rice and buckwheat.

Both rice and buckwheat are carbohydrates containing little if any lignin.

Applicants several pages from "Roempp", a standard chemical encyclopedia in German to support this assertion.

Page 1, second paragraph from "Roempp" lists the composition of rice:
100g of unpolished rice contains on average

13.1 g water,
7.4 g proteins,
2.4 g lipids,
75.4 carbohydrates,
0.67 crude fiber,
1.2 g mineral nutrients and B vitamins.

Thus, lignin is not a significant component of rice.

Buckwheat, is a purer carbohydrate substrate than lignocelllulosic materials. Buckwheat belongs to the carbohydrates based on starch. Applicants attach a page from "Roempp" same standard chemical encyclopedia in German giving the composition of buckwheat. See paragraph 2, titled "Zusammensetzung:".

The composition of 100 g buckwheat flour contains on average
6.3 % proteins,
1.1% lipids,
79.7% carbohydrates with 0.5% fibers, and
12% water.

Therefore, lignin is not a significant component of buckwheat and Minowa's stillage streams contain no significant lignin component.

In summary:
The combination of Brink with Minowa does not suggest or teach a process for separating suspended solids from a fermentation liquor by subjecting the liquor to a solids-liquid separation stage,
wherein the fermentation liquor is produced in a fermentation process for the production of a fermentation product,
which liquor comprises water, lignin and BOD,
by adding component ii) AND at least one component selected from the group consisting of (iii), (iv), (v) and (vi).

Further as Brink removes essentially all of the lignin before fermentation and Minowa's stillage stream contains essentially no lignin, neither references suggests the flocculation of suspended solids containing lignin.

**New Claim 20**
In regard to new claim 20, clearly the suspended solids of Brink and Minowa do not contain mainly lignin (i.e. >50% lignin).

**Claims 4-6, 8-12 and 14-16 are rejected under 35 USC 103(a) as being unpatentable over Brink US 5,536,325 in view of Minowa and further in view of Hughes US 6,967,085.**

Hughes,US 6,967,085 is directed to a process of flocculating microbial cell material from suspending media, such as a fermentation broth, using a cationic polymer (low IV) and a non-ionic or cationic polymer (high IV) (col. 2, lines 47-56). As the fermentation broth is derived from carbohydrate substrates such as starch or corn meal (cf. col. 3, lines 32-40) and not from lignocellulosic materials, the fermentation liquor of Hughes does not contain any lignin material, in contrast to the fermentation liquor of the present application which contains lignin.

The Applicants support the assertion that the carbohydrate substrates referenced in Hughes such as starch, corn meal etc. do not contain significant amounts of lignin material by submitting a section from Ulmann's Encyclopedia which discusses the makeup and composition of various fermentable

carbohydrates. This reference clearly distinguishes sugar crops and starches such as corn and potato from lignocellulosic materials. See section 5.4.3, page 12.

Applicants further support this assertion (the absence of lignin from the carbon sources of Hughes) by including two references cited in Hughes in col. 1, lines 23-25 and 37-38. In particular, Hughes states that Sitkey et al and Mukhopadhyay et al in Biotechnolgy Techniques teach the removal of solids from a fermentation broth. The applicants have read both cited references, and enclose both references for the examiner's convenience and information. Mukhopadhyay considers several carbon sources for the fermentation broth, none of which are based on lignocellulose. See table 2, page 123. Sitkey uses a complex fermentation medium containing potato starch, corn meal, casein, corn steep liquor, fodder yeast and diammonium phosphate. See page 50 of Sitkey.

It is clear that the fermentation liquors of Sitkey and Mukhopadhyay do not contain suspended solids containing lignin. It is also clear that Hughes' carbon sources are not lignocellulosic derived.

As a result applicants aver that Hughes does not suggest the flocculation of suspended solids containing lignin from the fermentation liquor.

As discussed above, the process according to claim 1 is unobvious in view of Brink and Minowa. The addition of Hughes, does not make up for the lack of suggestion in Brink and Minowa's to flocculate suspended solids containing lignin because Hughes also does not suggest the flocculation of suspended solids containing lignin.

Hence, the subject-matter of claims 4-6, 8-12 and 14-16 is unobvious in light of Brink, Minowa and Hughes.

**In regard to new claim 20:**

In regard to new claim 20, clearly the suspended solids of Brink, Minowa and Hughes do not contain mainly lignin (i.e. >50% lignin). Additionally, none of these references suggest that the suspended solids of the fermentation liquor be mainly lignin. Thus this claim cannot be obvious in light of the cited references.

**Claim 7 is rejected under 35 USC 103(a) as being unpatentable over Brink US 5,536,325 in view of Minowa and further in view of Moffett US 6,132,625.**

Moffett, US 6,132,625 relates to a process of separating biosolids from an aqueous stream resulting from animal or vegetable processing operations using an anionic inorganic colloids and a cationic polymer having a molecular weight greater than 1,000,000 (cf. claim 1) as flocculants.

The flocculants in Moffett are added to biosolids containing aqueous stream. Moffett teaches that the aqueous stream to be treated can be from any processing plant that produces an aqueous stream comprising biosolids, such as food processing plants. For example, animal slaughterhouses and animal processing plants and other food processing plants may produce aqueous streams comprising protein, fats and oil. .... Other food processing plants include plants for vegetable, grain and dairy food processing, for example, plants for processing soybeans, rice, barley, cheese, and whey; plants for wet-milling of starches and grains; as well as breweries, distilleries and wineries. See col. 3, lines 5-21.

The reference to breweries, distilleries and wineries does not even remotely suggest or teach the flocculation of suspended solids containing lignin in a fermentation liquor. And certainly, does not come close to teaching flocculation of suspended solids containing maily lignin according to claim 20.

The process according to claim 1 requires the flocculation of suspended solids containing lignin in a fermentation liquor. As suggested above, this step is not suggested by Brink, Minowa or Hughes. Moffett does not make up for this deficiency as the Moffett reference to breweries, distilleries and wineries and treatment of biosolids generation therefrom, is too vague and indefinite to suggest the specific step of applying flocculants to a fermentation liquor comprising lignin. As indicated in the enclosed Ulmann's encyclopedia, fermentable carbohydrates vary considerably. Lignocellulosic is only one of many possible alcohol resources.

Hence, the subject-matter of claim 7 is not rendered obvious by Brink, Minowa and Moffett.

**Claim 18 is rejected under 35 USC 103(a) as being unpatentable over Brink 5,536,325 in view of Minowa and further in view of Chieffalo, US 5,975,439**

As already mentioned in the prior art of the present application Chieffalo et al. (US 5,975,439) relates to an automated process for producing ethanol shredding the cellulosic component of municipal solid waste and mixing this with equal amounts of concentrated sulphuric acid to provide a hydrolyzed

mixture. At this stage, the solid by-product containing lignin is separated by filtration and the hydrolysate is subjected to fermentation (cf. col. 10, lines 49-53; col. 6, lines 40-49). Therefore, the fermentation liquor does not contain any lignin which may be separated and dewatered according to the present process.

Also the combination of the references above does not arrive at the particular required treatment system added to the fermentation liquor. For example, Minowa adds only coagulants. The present claim requires the addition of a high intrinsic viscosity cationic polymer (of at least 4 dl/g). Brink, Minowa or Chieffalo does not suggest such an addition.

In a conclusion, claim 1 and its dependent claims are unobvious in view of the cited documents.

**Double Patenting Rejections**

Applicants respectfully request that they be allowed to delay the submittal of terminal disclaimers in light of the claims of 10/587,582 and 10/587,583 until the other rejections have been resolved. At that time, both the Office and applicants will know the scope of the actual claims to be granted.

Reconsideration and withdrawal of the rejection of claims 1-19 is respectfully solicited in light of the remarks and amendments *supra*.

Since there are no other grounds of objection or rejection, passage of this application to issue with claims 1-20 is earnestly solicited.

Applicants submit that the present application is in condition for allowance. In the event that minor amendments will further prosecution, Applicants request that the examiner contact the undersigned representative.

Respectfully submitted,

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Enclosures: Request for Continued Examination, Section of Ullman's Encyclopedia on Fermentable Carbohydrates, two sections taken from *Roempp* (German chemical dictionary) defining the contents of Rice (Reis) and Buckwheat (Buchweizen), Sitkey V. et.al, *Biotechnology Techniques*, Vol. 6, No. 1, 49-52 (1992) and Mukhopadhyay A. et. al., *Biotechnology Techniques*, Vol. 4, No. 2, 121-126 (1990).

### 5.4. Raw Materials and Processes

Raw materials for the production of ethanol by fermentation can be classified as:

1. readily fermentable carbohydrates that can be used directly, and
2. starch and other organic materials that must be converted to a fermentable form prior to fermentation.

The raw materials come from three major sources:

1. agricultural crops,
2. forest products, and
3. industrial and agricultural byproducts and residues.

Depending on need, end use, and availability, the choice of raw material varies for different regions, countries, and industries.

#### 5.4.1. *Readily Fermentable Carbohydrates*

Various sugar crops, such as sugarcane, sugar and fodder beet, fruit crops, and crops based on crassulacean acid metabolism (CAM), are in this category.

***Sugarcane.*** Sucrose [57-50-1] ( α-D-glucopyranosyl- β-D-fructofuranoside) is the sugar obtained from cane or beet ( ⇒ Sugar). Sugarcane is a tropical crop whose successful cultivation is limited to an area spanning 37 °N to 31 °S.

Although sugarcane is grown mainly for production of table sugar and molasses, it is also an excellent raw material for the production of ethanol. The fermentable carbohydrates from sugarcane can be used either as the cane juice directly or as blackstrap molasses (a sugar byproduct). A material balance shows that 160 kg of fermentable solids can be obtained from 1 t of cane [263].

The cane juice is prepared by crushing raw cane and extracting the sugar with water, followed by clarification using milk of lime and $H_2SO_4$ to precipitate the inorganic materials [264]. The resulting extract is a green, sticky fluid, slightly more viscous than water, with an average sucrose content of 12 – 13 % [265].

Blackstrap molasses is the residue remaining after sucrose has been crystallized from cane juice. Molasses is a heavy viscous material, which contains sucrose, fructose, and glucose (invert sugar) at a total concentration of ca. 50 – 60 % (wt/vol) [266]. In contrast to cane juice, molasses is stable on storage and is usually diluted to the desired concentration just prior to fermentation.

A typical process for the production of ethanol from sugarcane is depicted in Figure 20. Production data are listed in Table 16. Ethanol production reaches a maximum after 14 – 20 h and then decreases until ca. 95 % of the available sugar is consumed. The process is usually batchwise, but some semicontinuous [232] and continuous operations [242], [267-269] are also used.

**Table 16. Yields in production of ethanol from sugarcane [267]**

| | Alcohol, indirectly from molasses | Alcohol, directly from sugarcane juice |
|---|---|---|
| Sugarcane yield in 1.5 – 2-year cycle (south-central region), t/ha | 63 | 63 |
| Average sucrose yield (13.2 wt %), | 8.32 | 8.32 |

| | | |
|---|---|---|
| t/ha | | |
| Crystal sugar production, t/ha | 7.0 | |
| Final molasses or cane juice production, t/ha | 2.21 | 66.2 |
| Fermentable sugar, molasses, or juice, t/ha | 1.32 | 8.73 |
| Alcohol yield at 100 % global efficiency, kg/ha | 675 | 4460 |
| Alcohol yield at reasonable 85 % global efficiency, L per ton of cane | 11.5 | 75 |
| or in L/ha | 730 | 4800 |

* Hectare (ha) = $10^4$ $m^2$.

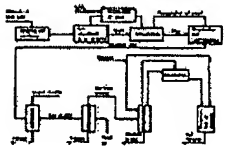


**Figure 20.** Typical process for the production of ethanol from sugarcane [267]

In the *batch process*, several fermentors are usually operating at staggered intervals to provide a continuous feed to the distillation columns. Overall productivity is ca. 18 – 25 kg of ethanol per cubic meter of fermentor volume per hour [225]. The "Melle Boinot" process is used in most Brazilian distilleries (see Section Batch Processes).

Ethanol has been produced in a *continuous process* (using continuously stirred tank reactors) from molasses by Danish Distilleries [268]. The process is shown in Figure 21, and performance data are given in Table 17.

**Table 17. Performance data for the Danish Distilleries process * [268]**

| | Fermentor 1 ($f_1$) ** | Fermentor 2 ($f_2$) ** |
|---|---|---|
| Amount of yeast and dry matter per liter, g | 10 | 10 |
| pH | 4.7 | 4.8 |
| Alcohol, vol % | 6.1 | 8.4 |
| Residual sugar, % | 1.0 | 0.1 |
| Temperature, °C | 35 | 35 |

* Residence time in each fermentor: 10.5 h; influx: 600 kg of molasses diluted in $22 \times 10^3$ L/h.
** See Figure 21.

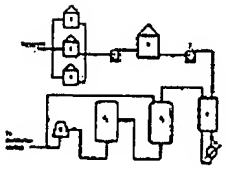


**Figure 21.** Continuous production of ethanol by Danish Distilleries [268]

a) Storage tank; b) Intermediate container; c) Metering pump; d) Regeneration section; e) Plate heat exchanger; f) Fermentor; g) Yeast separator

According to this process, the molasses is stored in two or three 1500-$m^3$ tanks from which it is pumped to intermediate containers. The material is adjusted for pH and nutrients (nitrogen and

phosphorus), sterilized at 100 °C by using plate heat exchangers, and then introduced to three fermentors with a total volume of 170 $m^3$. The fermented wort is centrifuged after fermentation, and the live yeast returned to the first fermentor. At the start, sufficient yeast propagation must be accomplished by aeration (0.02 – 0.03 L of air per liter of liquid per minute). The yield is ca. 28.29 L of alcohol per 100 kg of molasses, or a maximum of ca. 65 L of alcohol per 100 kg of fermentable sugar.

A continuous process for production of beer from sugar by use of *tower fermentors* is shown in Figure 22 [270], [271].

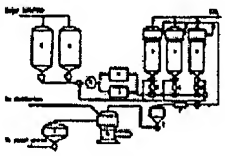


**Figure 22.** Commercial ethanol tower fermentation system (APV Company) [242]

a) Buffer storage tank; b) Divert line; c) Pasteurizer; d) Flow controller; e) Vertical cylindrical tower; f) Chiller; g) Yeast green beer buffer storage; h) Centrifuge; i) Separator

The key to the process is a vertical cylindrical tower fermentor with a conical bottom. A baffled yeast settling zone constitutes the upper part of the fermentor. The fermentor uses a flocculent yeast, which is pumped into the base of the tower. As the reaction proceeds, the beer rises, and the flocculating yeast settles and is retained in the reactor. High cell densities of 50 – 60 g/L are achieved without the use of mechanical cell concentration or separation devices. Short residence time (<4 h) with a sugar concentration of up to 12 % (wt/vol) sucrose, 90 % sugar utilization, and 90 % conversion to ethanol, produce up to 5 % ethanol in the final broth. The overall productivity of this system can be up to 80 times higher than that of the simple batch system.

***Sugar Beet.*** Like sugarcane, sugar beet produces carbohydrates that consist primarily of sucrose ( ⇒ Sugar). Sugar beet is a more versatile crop than sugarcane. It can tolerate a wide range of soil and climatic conditions, and is grown throughout nearly half of the United States, Europe, Africa, Australia, and New Zealand.

In addition to sucrose, sugar beet contains sufficient nitrogen and other organic and microorganic nutrients [272] so that little, if any, fortification is required prior to fermentation. Another benefit is the high yield of coproducts such as beet tops and extracted pulp. The pulp has a high feed value, and the tops may be returned to the soil for erosion control and nutrient replacement. The yield of fodder beets is high (ca. 50 – 150 t/ha); their composition is described in [273].

A new fodder beet crop, produced in New Zealand through a genetic cross between sugar beets and marigolds, gives greater yields of fermentable carbohydrates per hectare than does sugar beet [274]. In addition, the sugar from fodder beet is reported to be more resistant to degradation over long storage.

Processes for the production of alcohol from sugar and fodder beets are basically the same as from sugarcane.

***Fruit Crops.*** Many crops (grapes, plums, peaches, apricots, pineapples, etc.) contain variable proportions of sugars (sucrose plus fructose, usually 6 – 12 %). The fruit sugars can be readily fermented to alcohol, and this is done on a large scale for production of alcoholic beverages. The alcohol content of the product, which basically is the liquid after fermentation, separation of yeast, further treatment, and aging, depends on use and fermentation conditions. Table wines have <14 % alcohol, whereas wines with >14 % alcohol fall in the category of desert wines and aperitifs ( ⇒ Wine). Higher concentrations of alcohol are achieved by means of distillation to produce "strong" alcoholic beverages (e.g., brandy, whiskey, gin, vodka) ( ⇒ Spirits).

Alcohol for industrial or fuel use is seldom produced from fruit and vegetable crops. However, some fruit from tropical and semiarid climates, such as dates [275], mohwa flowers [276], and rain tree fruit [277], have been investigated for fuel alcohol production.

***Crops Based on Crassulacean Acid Metabolism.*** Interest in using the agriculturally semi- or nonproductive regions of the world to grow alcohol-producing crops has increased [278]. These regions could be used to grow plants that utilize crassulacean acid metabolism (CAM) because their photosynthetic metabolism is extremely efficient with respect to irrigation requirements. These plants exhibit above-average productivity (expressed as a function of biomass production per unit of existing biomass) compared to other agricultural crops.

The CAM plants that are high in fermentable carbohydrates include various cacti (e.g., *Opuntia* sp.) and other plants such as *Euphorbia lathyrus* and *Agave* sp. Few data are available on potential ethanol production from these crops and its economic feasibility; however, an estimated 50 t/ha of these crops could be produced annually in subagricultural areas [279].

### 5.4.2. *Starch*

A variety of starch materials, such as grains, cassava, sweet potatoes, sweet sorghum, and Jerusalem artichoke, can be used for fermentation to ethanol ( ⇒ Starch). Selection depends on various factors, the major ones being climate and availability for large-scale production. Corn, wheat, potatoes, and Jerusalem artichokes are the most common raw materials in Europe and North America, whereas rice, cassava, sweet potato, and sweet sorghum are important in tropical countries.

***Corn.*** Corn is the preferred raw material for conversion to alcohol in the United States and parts of Europe. It is available in large quantity, and its price (especially for low-grade or distressed corn) is thus acceptable for conversion to ethanol. Conversion to ethanol is efficient, and byproducts, such as corncobs, stalks, and leaves, are valuable as animal feed, energy source, or fertilizer. About 66 % of corn production is used for food and feed, and ca. 5 % is used to make alcohol ( ⇒ Cereals – Maize (Corn)).

A number of batch and continuous processes have been developed for production of ethanol from corn. A conventional fermentation plant producing $76\times10^3$ $m^3$ of anhydrous ethanol per year from $816.5\times10^3$ kg of corn per day is shown in Figure 23 .

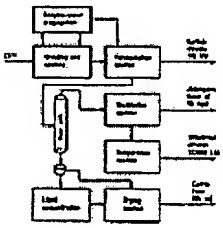


**Figure 23.** Flow diagram for a conventional fermentation plant producing anhydrous ethanol from corn [280]

In this process, corn is ground and cooked to dissolve and gelatinize the starch. The enzymes α-amylase and glucoamylase are then added to hydrolyze the starch to fermentable monosaccharides. After yeast fermentation for ca. 48 h at 32 °C, about 90 % of the starch is converted to ethanol. The fermentation broth is fed to the beer still where alcohol (ca. 50 vol %) is distilled. Subsequent distillation produces 95 % alcohol, which can be further concentrated by azeotropic distillation using benzene. After centrifugation, the stillage is concentrated to ca. 50 % solids in a multiple-effect evaporator, further concentrated in a fluidized-bed, transport-type dryer to ca. 10 % moisture, and then used as such for animal feed. This feed contains all the protein originally present in the grain, plus the additional protein from the yeast, resulting in a product containing 28 – 36 wt % protein.

In addition to alcohol and cattle feed, the original $816.5\times10^3$ kg of corn yields $175\times10^3$ kg of $CO_2$ and 95 kg of byproduct aldehydes, ketones, and fusel oils.

Alltech developed a method for an integrated grain-processing – fermentation route [281]. The grain pretreatment step prior to fermentation is shown in Figure 24.

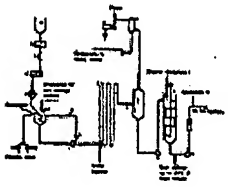


**Figure 24.** Alltech process for continuous whole mash cooking [281]

a) Grain hopper; b) Screen; c) Magnets; d) Continuous weigher; e) Hammer mill; f) Slurry vessel with agitator, temperature 50 – 70 °C; g) Rupture disk; h) Expansion vessel; i) Positive displacement pump; j) Continuous cooker tube, residence time 5 min, temperature variable up to 150 °C; k) Pressure valve; l) Flash vacuum cooler to 66 – 76 °C; m) Condenser; n) Open impeller pump; o) Converter, residence time 20 min, agitator 1 rpm; p) Wort cooler

Two enzymes, alcoholase I (from *Bacillus subtilis*) and alcoholase II (from *Aspergillus niger* and *Rhizopus niveus*) are used to hydrolyze the starch to fermentable sugars. Continuous whole mash cooking is applied. The ground starch is first mixed with water and alcoholase I at 60 °C, and then cooked at 93 – 165 °C in a batch or continuous cooker. The cooked mash is then cooled to ca. 66 – 76 °C, and a second portion of alcoholase I is added; 20 min is allowed for conversion. After this first hydrolysis step, the temperature is adjusted to 32 °C and the mash, supplemented with alcoholase II, is fermented with yeast.

***Cassava.*** Cassava, also known as manioc, mandioc, aipum, yuca, cassada, and tapioca, is second in importance only to the sweet potato as a root crop throughout the tropics and in parts of South America where the plant originated. It was taken to West Africa by the Portuguese around 1914, where it now seems to have replaced yams and cocoyams because it adapts easily and requires less labor than other crops. Cassava is one of the highest yielding plants of the vegetable kingdom (10 – 30 t/ha ); it requires little cultivation and the tubers can be left in the ground until required without serious deterioration.

Cassava (genus *Manihot*) is in the family Euphorbiaceae, which belongs to the subdivision Angiospermae, class Dicotyledoneae, order Geraniales. This large, widely spread family comprises 283 genera including 7300 species, with an almost worldwide distribution [282].

*Manihot esculenta*, *M. utilissima,* and *M. dulcis* are some economically important members of a genus which includes over 150 species that are distributed throughout tropical countries. The species include herbs, shrubs, and trees, many of them producing latex and some yielding rubber. Brazil, Indonesia, and Zaire are the largest producers of cassava.

Roots are generally of interest for alcohol production. They contain 20 – 35 wt % starch and 1 – 2 wt % protein, although strains with up to 38 % starch have been developed [283]. The advantages of cassava for fuel ethanol production (which can amount to 7600 L/ha) have been assessed [267], [284], [285]. The process used to obtain ethanol from cassava is shown in Figure 25.

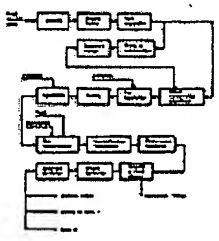


**Figure 25.** Production of ethanol from cassava root [267]

Fresh roots are washed, peeled, and ground into a mash. Part of this mash is dried; it can be stored in this form up to a year and is used for animal feed. For fermentation to ethanol, the starch is hydrolyzed with α-amylase, which is added in two steps. The first addition decreases the vicosity of the mash and facilitates cooking. In the second addition, the enzyme completes liquefaction of the starch. After that glucoamylase is added, which converts the liquefied starch to glucose and prepares the mash for fermentation. The fermentation process is the same as the one used for production of alcohol from sugarcane.

Alcohol yield from cassava is 165 – 180 L/t, which, on a mass basis, is higher than that obtained from sugarcane [285]. However, because sugarcane production can be as high as 90 t/ha, the alcohol yield per unit area is greater from cane under present cultivation conditions. Another advantage of cane is its dry fiber content, which equals the amount of total sugar present. This amount of fiber (bagasse) is sufficient to maintain the energy requirements of the plant; this is not the case with cassava, which only contains ca. 3.5 % fiber. Another disadvantage of cassava is that it does not contain readily fermentable sugars and, therefore, requires considerable processing of the roots prior to fermentation.

***Sweet Sorghum.*** Sweet sorghum (*Sorghum sacchartum*) contains both starch and sugar. Its yield of ethanol from fermentable sugars is ca. 3500 – 4000 L/ha; an additional 1600 – 1900 L/ha can be produced from stalk fibers. There are more than 17 000 varieties of sorghum, and the yield is anticipated to increase by 30 % with some new hybrids [286]. The plant is adaptable to most of the world's agricultural regions; it is resistant to drought, and its nutrients are efficiently utilized by animals.

The fermentable sugars and starches are treated conventionally for ethanol production. The free sugars are fermented directly, whereas the starches are hydrolyzed by use of amylases, as is the case with cassava.

***Potato.*** The potato is a common starch crop worldwide ( ⇒ Starch). The potato originated in South America (Chile and Peru) and came to Europe through Spain at the end of the 16th century. It is now grown in almost all climates and almost all types of soil, including dry and sandy soil [287].

Starch is the main carbohydrate component of potato (ca. 68 – 80 %). Depending on cultivation and variety of potato, starch content can vary between 12 and 21 % in raw potatoes. Only small quantities of soluble sugars are present (0.07 – 1.5 % sucrose, glucose, and fructose), as well as some rubber and dextrins (0.2 – 1.6 %) and pentosans (0.75 – 1.00 %).

The production of ethanol is based on fermentation of the available starch. A process developed by Danish Distilleries is shown in Figure 26 [268]. The process is semicontinuous and is applicable to both potatoes and grain.

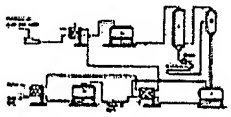


**Figure 26.** Danish Distilleries semicontinuous production of alcohol from potatoes or grain [268]

a) Preheater; b) Pulper; c) Enzyme treatment vessel; d) Flash cooler; e) Boiler tube; f) Holding tank; g) Condenser; h) Liquefaction vessel

Potatoes are mashed and then treated with amylases to hydrolyze the starch. The treatment section involves rapid steam treatment at 150 °C for ca. 3 min. The mash is cooled to 70 °C for liquefaction with commercial amylase preparations of bacterial origin; it is then cooled further to 30 °C and used for alcohol fermentation in the customary manner.

***Jerusalem Artichoke.*** The Jerusalem artichoke (*Helianthus tuberosus*) is a member of the Compositae family and is closely related to the sunflower (*Helianthus annus*), earning it the nickname "wild sunflower". About 102 different names are synonymous with the name *H. tuberosus*.

The plant is native in North America. It was originally grown by the Cree and Huron Indians who called it askipaw and skibwan, respectively. The plant was introduced to Europe at the beginning of the 16th century where it rapidly spread through the Mediterranean countries. The addition of "Jerusalem" to the name is most likely the result of an English version of *Girasole*, the Italian name for this plant [288].

The plant grows 1.5 – 2.5 m tall for wild strains and up to 3.7 m under cultivation. Top growth accounts for 40 – 56 % of the total plant biomass. The tubers are of greatest interest as a raw material for fermentation to ethanol.

The main soluble carbohydrate in the Jerusalem artichoke is inulin, which is composed of a homologous series of polyfructofuranose units. These units consist of linear chains of D-fructose molecules joined by β-2,1-linkages. The chains are terminated by a D-glucose molecule linked to fructose by an α-1,2-bond as in sucrose [289].

A process to produce $360\times10^{3}$ kg/a of ethanol from Jerusalem artichoke tubers is presented in Figure 27 [290].

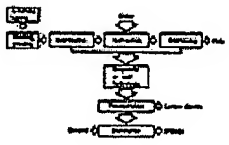


**Figure 27.** Production of ethanol from Jerusalem artichoke tubers [290]

In this process, the juice is expressed from the tubers and extracted with water to obtain a carbohydrate concentration of about 20 %. The carbohydrates (predominantly inulin) are then hydrolyzed enzymatically by activating the endogenous inulinases at ca. 50 – 60 °C; acid hydrolysis (pH ca. 1) of inulin is also effective. The resulting fermentable sugars are then converted to ethanol by a conventional route.

Novel routes for conversion of the juice to ethanol have also been explored; the flocculating yeast *Saccharomyces diastaticus* has been used in semicontinous and continuous modes [234].

### 5.4.3. *Lignocellulosic Materials*

Lignocellulose is the largest terrestrial source of biomass that is renewably produced through photosynthesis ( ⇒ Biomass Chemicals; ⇒ Cellulose – Cellulose; ⇒ Lignin). The solar energy reaching the earth surface is $3.67\times10^{21}$ kJ/a [291]. Gobal photosynthesis (with an efficiency of 0.07 %) could convert $2.57\times10^{18}$ kJ of that energy to cellulose-containing biomass. This would result in a net production of $1.8\times10^{11}$ t/a of biodegradable material, 40 % of which is cellulose [292]. Estimates are that $1 - 1.25\times10^{11}$ t/a of terrestrial dry mass is produced together with $0.44 - 0.55\times10^{11}$ t/a in the oceans [293]. Present removal of this potential energy source is ca. 0.5 % of the total growing stock on a global basis [294].

The fermentation potential of lignocellulose is based mainly on the cellulose content of the biomass. Chemically, cellulose is similar to starch. It is a polymer of glucose in which the glucose units are linked by β-1,4-glucosidic bonds, whereas the bonds in starch are predominantly α-1,4-linkages. The degree of polymerization (DP) varies for different sources of cellulose; for example, newsprint cellulose has a DP of 1000, whereas cotton has a DP of ca. 10 000 [295].

The cellulose molecule is more resistant to hydrolysis compared to starch. This resistance is due not only to the primary structure based on glucosidic bonds, but also, to a great extent, to the secondary and tertiary configuration of the cellulose chain, as well as its close association with other protective polymeric structures such as lignin, starch, pectin, hemicellulose, proteins, and mineral elements.

The lignin molecule seems to be primarily responsible for difficulties in hydrolyzing the lignocellulosic material, because it forms a protective sheath around the cellulose microfibrils. Lignin is a macromolecule of phenolic character and can be viewed as a dehydration product of three monomeric alcohols: *trans*-4-coumaryl alcohol, *trans*-coniferyl alcohol, and *trans*-sinapyl alcohol. The relative amount of each varies with the source [296].

When cotton cellulose is treated with dilute acid, partial hydrolysis occurs rapidly, and ca. 15 % of the cellulose chain is degraded to glucose. The remaining 85 % is more resistant to hydrolysis, possibly because this portion of the cellulose exists in a highly crystalline order [295].

In order to use lignocellulosic materials for fermentation to ethanol, they must be pretreated and then hydrolyzed to fermentable sugars. Pretreatment may be physical or chemical, e.g., milling, steam explosion, or use of solvents and various swelling agents.

In *steam explosion* green wood chips are heated to ca. 180 – 200 °C for 5 – 30 min in a continuous operation (Stake process), or to a temperature of 245 °C for 0.5 – 2 min (Iotech process) [297]. The acids formed from hemicellulose under these high-temperature and high-pressure conditions start to "autohydrolyze" the cellulose and intact lignin. Lignin is sufficiently softened at the end of the steaming period, so that when the vessel is suddenly depressurized to atmospheric pressure, an explosion occurs within the woody cells. This partially disrupts the close association of cellulose with lignin and consequently increases the surface area available for further hydrolysis. The effect of steam pretreatment on the enzymatic hydrolysis of various cellulose-containing materials is shown in Table 18.

The pretreated lignocellulosic material is then subjected to further hydrolysis, which can be acidic or enzymatic ( ⇒ Cellulose – Cellulose; ⇒ Enzymes – Cellulases). A comparison of enzymatic and acid hydrolysis for cellulose degradation is given in Table 19. Cellulose and its degradation products are the only materials considered for fermentative purposes.

**Table 18. Effect of steam pretreatment on the enzymatic hydrolysis [*] of cellulosic substrates [298]**

| Substrate | Pretreatment | Total reducing sugars, mg/mL | |
|---|---|---|---|
| | | 4 h | 24 h |
| Hardwoods | | | |
| Poplar | none | 1.4 | 2.4 |
| | steam | 15.3 | 25.8 |
| Aspen | none | 1.8 | 3.0 |
| | steam | 12.8 | 24.8 |
| Agriculture residues | | | |
| Corn stover | none | 4.9 | 7.8 |
| | steam | 15.7 | 22.5 |
| Sugarcane bagasse | none | 1.7 | 2.5 |
| | steam | 9.5 | 16.1 |
| Urban waste | none | 10.5 | 18.0 |
| | steam | 6.2 | 10.8 |
| Softwoods | | | |
| Eastern spruce | none | 2.0 | 3.8 |
| | steam | 3.5 | 6.4 |
| Douglas fir | none | 1.6 | 3.2 |
| | steam | 2.8 | 4.3 |

[*] *Trichoderma reesei* cellulase (QM9414), 19 IU (International Units) per gram of substrate; 5 % substrate slurries, pH 4.8, 50 °C; steamed substrates washed prior to enzymatic hydrolysis.

**Table 19. Comparison between enzymatic and acid hydrolysis of cellulosic materials [227]**

| Acid | Enzyme |
|---|---|

| | |
|---|---|
| Nonspecific catalyst; therefore, will delignify material as well as hydrolyze cellulose | Specific macromolecular catalyst; therefore, extensive physical and chemical pretreatment of material necessary to make cellulose available for degradation |
| Decomposes hemicellulose to inhibitory compounds (i.e., furfural) | Produces clear sugar syrup ready for subsequent anaerobic fermentation |
| Harsh reaction conditions necessary; therefore, increased cost of heat- and corrosion-resistant equipment | Run under mild conditions (50 °C, atmospheric pressure, pH 4.8) |
| High chemical cost requires catalyst recovery and reuse | Cost of producing cellulases is the most expensive process step; therefore, recycle is necessary |
| High rate of hydrolysis | Lower rate of hydrolysis |
| Low overall yield of glucose because of degradation | High glucose yield depending on system and pretreatment |

An example of a semicontinuous process for ethanol production from wood is shown schematically in Figure 28; this process uses dilute sulfuric acid for cellulose prehydrolysis.

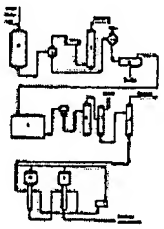


**Figure 28.** Ethanol production from wood [299]

Optimum conditions for this process are: acid concentration in total water, 0.53 %; maximum temperature of percolation, 196 °C; rate of temperature rise, 4 °C/min; percolation time, 145 –190 min; ratio of total water to oven-dried wood, 10; percolation rate, 8.69 – 14.44 L $min^{-1}$ $m^{-3}$

a) Digester; b) Flash evaporator; c) Furfural tower; d) Neutralization vessel; e) Clarifier; f) Fermentor; g) Yeast separator; h) Alcohol stripper; i) Extraction tower; j) Rectifying tower; k) Evaporator; l) Vapor compressor

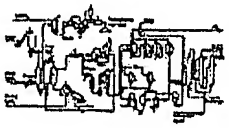
The hydrolysate percolates through a bed of wood chips. Optimum conditions for the process are described in [299]. After acid digestion (a), the effluent passes through a flash evaporator (b), which separates the vapors containing furfural and methanol from the underflow condensate containing the sugar solution. The acid hydrolysate solution is further neutralized with a lime slurry, and the precipitated calcium sulfate is separated in a clarifier (d) as a 50 % solids sludge. The neutralized liquor is blended with recovered yeast (*Saccharomyces cerevisiae*) from previous fermention and is fermented to ethanol (e), which is further concentrated to 95 % by distillation (i).

The bottom material from the alcohol stripping column (g), which contains pentose sugars, is further concentrated in multiple-effect evaporators (j) to a 65 % solution, that can be used as animal feed or for chemical conversion to furfural.

Figure 29 shows a strong acid hydrolysis process.

**Figure 29.** Ethanol production from wood by use of strong acid hydrolysis [300]

a) Feed hopper; b) Feeder; c) Digester; d) Neutralization vessel; e) Multiple-



effect evaporators; f) Dryer; g) Electrodialysis membrane; h) Filter; i) Fermentor; j) Carbon dioxide scrubber; k) Seed fermentor; l) Centrifuge; m) Yeast wash vessel; n) Surge vessel; o) Beer still; p) Alcohol column

The air-dry wood is first pretreated with dilute sulfuric acid (c). Complete hydrolysis is accomplished in a subsequent strong acid cycle in which cellulose is hydrolyzed at room temperature with 70 – 80 % $H_2SO_4$. The glucose, retained by the dialysis membrane (g), is neutralized, deionized, and then sent to fermentation (i). The sulfuric acid permeate from the dialysis unit is evaporated and reconcentrated for recycle. Lignin is separated from the concentrated acid by filtration (h) and washing.

To illustrate enzymatic hydrolysis of cellulose for alcohol production, a process used by the Natick Development Center is shown in Figure 30. A large part of this process involves the preparation of the cellulase enzyme. Newspaper is the cellulose-containing substrate.

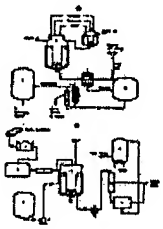


**Figure 30.** Enzymatic hydrolysis of newsprint by Natick Development Center (NDC) [298]

A) Pilot plant process for cellulase production:

a) Production vessel (vertical filters); b) Inoculum vessel; c) Filter; d) Harvest storage; e) Ultrafilter; f) Concentrate storageB) Pilot-plant process for newspaper hydrolysis: a) Ball mill; b) Solids metering; c) Solids transfer; d) Bioreactor; e) Enzyme storage; f) Metering pump; g) Harvest pump; h) Crude filter; i) Polish filter; j) Evaporator

### 5.4.4. *Waste Materials and Residues*

Various types of agricultural, industrial, or municipal refuse and waste can be used as substrates for ethanol fermentation. The fermentation is based on available sugar, starch, or cellulose in the waste material. The major advantage of this route lies in coupling waste treatment with the production of a higher value product. Both environmental pollution abatement and process economics are thus improved.

***Cornstalks.*** Cornstalks are available in large quantities as a byproduct of corn agriculture. This material is predominantly composed of lignocellulose. A two-stage process using dilute acid treatment followed by concentrated acid impregnation of the lignocellulosic material is shown in Figure 31.

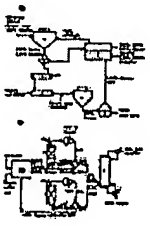


**Figure 31.** Production of ethanol from cornstalks [301]

A) Acid hydrolysis: a) Prehydrolysis tank, 4.4 % $H_2SO_4$; b) Filter; c) Rotary dryer; d) Impregnator; e) Hydrolysis tank, 8.0 % $H_2SO_4$; f) Filter; g) Electrodialysis unit

B) Fermentation: A) Acid hydrolysis process; h) Fixed film of *Fusarium oxysporum*; i) Centrifuge; j) Fixed film of *Saccharomyces cerevisiae*; k) Distillation column

In this process, ground corn stover (841 nm, 20 mesh) is treated with 4.4 % $H_2SO_4$ at 100 °C for 50 min (a). The mixture is then filtered (b) and the xylose-rich liquid is processed by electrodialysis (g) for acid recovery. The solids are dried further (c) and impregnated with 85 % $H_2SO_4$ (d), followed by dilution with water to give a $H_2SO_4$ concentration of 8 % (e).

Subsequent hydrolysis is carried out at 110 °C for ca. 10 min, and acid is again recovered by electrodialysis. The combined yield of xylose is 94 %, and the yield of glucose is 89 %. Glucose is converted to ethanol by *Saccharomyces cerevisiae* (j) and xylose by *Fusarium oxysporum* (h), both in immobilized cell reactors. The overall annual capacity of the plant is $17\times10^{3}$ $m^{3}$.

***Domestic Refuse.*** Domestic refuse contains a complex variety of materials that come mainly from cellulosic-type residues. A large quantity of this material, ca. 1.3 – 2.2 kg per person, is generated daily. A process for the production of 36.5 t/d of ethanol from domestic refuse is illustrated in Figure 32 [302].

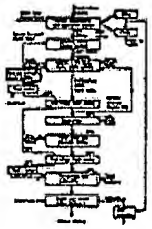


**Figure 32.** Flow diagram for continuous production of ethanol from refuse with 60 % content of cellulose [302]

* Biochemical oxygen demand.

The refuse is separated into dense and light fractions by the use of a flotation separator or a special pulper. The pulped fraction, which contains cellulose, is first subjected to removal of fines and plastics, and then introduced into a reactor where it is hydrolyzed with 0.4 % $H_2SO_4$ for ca. 1.2 min at 230 °C. This process is followed by flash cooling, neutralization with $CaCO_3$, and filtration. Fermentation of the sugar solution takes ca. 20 h at 40 °C and yields ca. 1.7 % aqueous ethanol solution, which is further concentrated by distillation to ca. 95 % ethanol.

***Waste Liquor from the Pulp and Paper Industry.*** Two chemical pulping methods are predominant in the pulp and paper industry: the sulfate (Kraft) and the sulfite processes ( ⇒ Paper and Pulp). The basis of the these operations is treatment of the lignocellulosic material (wood, straw, etc.) with highly concentrated acid or base, which should dissolve the lignin portion of the wood and leave cellulose fibers that are processed into the final paper product. Depending on conditions (temperature, pressure, concentration of chemicals, chemical to wood ratio, and time of digestion), more or less delignification and breakdown of the original cellulose occur. As a result, a product pulp is produced as well as a waste chemical liquor, which basically consists of spent cooking chemicals. The more drastic the delignification conditions (low yield process), the better is the quality of the paper obtained. The high-yield process refers to milder delignification and a pulp that still contains a considerable amount of lignin. Low-yield processes are characterized by waste liquors with a high concentration of chemicals and a higher organic content.

The organic content of these liquors is primarily sulfonated lignin (e.g., 43 % of organic dry substance in a spent spruce sulfite liquor). However, cellulose and hemicellulose are also partially hydrolyzed during digestion so that waste liquors contain a certain proportion of free sugars (hexoses and pentoses in ca. 2 – 4 % concentration and ca. 14 % total solids) [303].

Tremendous quantities of waste liquor are generated in a pulp and paper mill; they amount to ca. 9180 L per ton of pulp produced [304]. Consequently, a chemical pulping process with a medium capacity of 500 t/d of pulp produces $4.59\times10^{6}$ L/d of waste liquors. Release of this liquor into natural waters is prohibited because both organic and toxic pollution result.

The sulfate (Kraft) process is designed so that the majority of the waste liquor can be recycled and its organic value converted to energy by combustion in a specially designed steam boiler (recovery furnace).

Previously, the majority of pulping mills worldwide were sulfite mills. Because of economic and environmental problems, sulfite pulping is gradually being phased out and the process converted to sulfate pulping or modified in other ways. Recovery of chemicals in the sulfite process is not as feasible as in the Kraft process, so large quantities of waste sulfite liquors

(WSL) are discharged to the environment.

Because WSL contain fermentable sugars, these liquors have been used efficiently as fermentation substrates for alcohol production. The process is relatively old (1908 in Sweden) but is still in operation in some mills (e.g., the Ontario Paper Company, Canada). A typical process for fermentation of WSL is shown schematically in Figure 33.

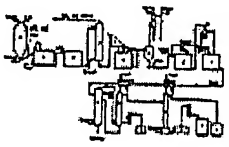


**Figure 33.** Production of ethanol from waste sulfite liquors (WSL) [305] *

a) Digester; b) Blowpits; c) Storage; d) Stripper; e) Screen; f) Storage; g) Flash cooler; h) Barometric condenser; i) Ejectors; j) Fermentor; k) Yeast separator; l) Storage; m) Preheaters; n) Beer still; o) Rectifying column; p) Oil washer; q) Fusel oil; r) Purifying column; s) Vaporizer; t) Condenser; u) Alcohol; v) Heads

* Reprinted with permission of American Institute of Chemical Engineers.

The waste liquor is first stripped of $SO_2$ with a conventional steam stripper. This is necessary because $SO_2$ would inhibit subsequent fermentation. The liquor is adjusted to give a ca. 10 – 12 % concentration of sugars, the pH is adjusted to 4.5, and nitrogen and phosphorus nutrient sources are added (e.g., urea and phosphate). The fermentation is conventionally carried out with yeast (*Saccharomyces cerevisiae*) at 30 °C for ca. 20 h. The yeast is usually concentrated and recycled, and the broth containing ethanol is sent to the distillation section.

***Cheese Whey.*** Whey is a byproduct of cheese production ( ⇒ Cheese, Processed Cheese, and Whey – Introduction, Processed Cheese, and Whey). An estimated $74\times10^6$ t of whey is produced annually worldwide. This amount of whey contains ca. $0.7\times10^6$ t of milk protein and 3.2 t of lactose [306].

Whey with its protein, carbohydrate, and vitamin content is a valuable, nutritious material; its composition is described in [307]. Whey is used in various forms as a component of either animal or human food. However, the utilization and recycling of whey nutrients depend on many factors, one of which is the size of the cheese factory. The smaller the factory, the less recycling of whey is practiced.

The alcohol produced from whey is derived mainly from the fermentation of available lactose. However, only a few microorganisms can convert lactose to ethanol, and conventional yeast (*Saccharomyces cerevisiae*) is not among them. The most efficient lactose-utilizing organism is reported to be *S. fragilis* [308].

A process using *Kluyveromyces fragilis* yeast was developed in Denmark (Fig. 34) [309-311]. The whey is first concentrated by reverse osmosis and ultrafiltration, and then introduced into fermentation vessels. The yield based on lactose is about 80 % of theoretical. About 42 L of whey, containing 4.4 % lactose, is required to produce 1 L of absolute ethanol.

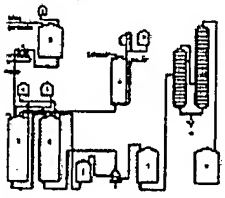


**Figure 34.** Continuous production of ethanol from whey [311]

a) Acid; b) Storage tank; c) Heat exchanger; d) Control; e) Antifoam; f) Chemicals; g) Fermentor; h) Substrate; i) Propagation plant; j) Storage; k) Separator; l) Buffer tank; m) Distillation; n) Alcohol storage

A better substrate for industrial fermentation of whey is enzymatically hydrolyzed lactose. β-Galactosidase-treated whey yields a mixture of monosaccharides, glucose, and galactose, which can be efficiently fermented by high-alcohol-producing yeasts [308], [312].

# Reis

Stand: Mai 2005 > Bearbeitet von: Stefan Gräber
Fachgebiet: Biotechnologie und Gentechnik > Unterthema: Landwirtschaft (Pflanzen)
Fachgebiet: Lebensmittelchemie > Unterthema: Getreide und Getreideprodukte, Backwaren

(*Oryza sativa* L.). Zu den Gräsern (Poaceae) zählende, in tropischen und subtropischen Regionen vielfach kultivierte Getreideart. Das Hauptanbaugebiet für Reis (ca. 90% der Weltproduktion) ist Südostasien (China, Indien, Indonesien, Vietnam u.a.), aber auch die USA, Mexiko, Brasilien, Westafrika und Südeuropa sind Produktionsgebiete. Im Anbau unterscheidet man Sumpf- oder Wasserreis sowie Trocken- oder Bergreis. Die Weltjahresproduktion liegt bei 546 Mio. t und dient ca. 2,2 Mrd. Menschen als Grundnahrungsmittel.

## Verarbeitung und Zusammensetzung:

Die nach dem Drusch vorliegenden bespelzten Körner (Roh- oder *Paddy-Reis*) werden zunächst entspelzt, wodurch man ernährungsphysiologisch wertvollen *Braunreis* erhält. Durch Schleifen und Polieren werden Frucht- und Samenschale (Silberhäutchen), der Keimling und die Aleuronschicht (vgl. Getreidekorn) entfernt. Diesen Reis bezeichnet man als *Weißreis*, wobei man je nach Korngröße und Form Rund-, Kurz-, Mittel- oder Langkornreis unterscheidet. 100 g unpolierter Reis enthalten durchschnittlich 13,1 g Wasser, 7,4 g Proteine, 2,4 g Lipide, 75,4 g Kohlenhydrate, 0,67 g Rohfaser, 1,2 g Mineralstoffe und B-Vitamine. Polierter Reis ist im Vergleich zu unpoliertem Reis sehr arm an Mineralstoffen und Vitaminen. Der ausschließliche Genuß von poliertem Reis führte bei Teilen der ostasiatischen Bevölkerung zur Beri-Beri-Krankheit (Thiamin-Avitaminose). Ein im Nährwert verbessertes Produkt wird durch den sogenannten Parboiling-Prozeß gewonnen.

Als wichtigste Aromakomponente von gekochtem Reis wurde 2-Acetyl-1-pyrrolin isoliert. Im Unterschied zu Europa und den USA sind in Asien Reissorten beliebt, die beim Kochen ein "popcornartiges" Aroma entwickeln. Es beruht auf der Bildung von 2-Acetyl-1-pyrrolin. Außer Vollreis oder Ganzreis (Ausbeute 45 –55%) fallen in der Reismüllerei Bruchreis bzw. Reismehl (20 –35%) und Spelzen bzw. Kleie (20 –24%) an.

## Verwendung:

Langkornreis wird für Parboiled-Reis, Schnellkochreis sowie Dosen- und Suppenreis verwendet; Kurz- und Mittelkornreis werden dagegen für Trockengetreideprodukte, für Babynahrung und zur Bierherstellung verwendet. Rundkornreis ist als Milchreis im Handel. Vollreis wird zur Herstellung von Puffgetreide (Puffreis) und insbesondere in Ostasien zur Herstellung von Wein (Sake) und Schnaps (Arrak) benutzt. Da Reis keine Zöliakie-auslösenden Proteine enthält, kann er zur Zubereitung von Gluten-freier Diät verwendet werden. Bruchreis wird zu Grieß, Mehl, Stärke oder zu Reispuder (für Kosmetika) verarbeitet. Aus Reiskleie wird hochwertiges Kraftfutter oder Keimöl hergestellt. Die beim Schälen anfallenden Spelzen dienen als Verpackungs-, Heiz- und Isoliermaterial.

## Gentechnik:

Reis besitzt ein kleines Genom (430 Mio. Basenpaare) und dient deshalb als Modellgenom für die Genomforschung an Getreide. Erfaßt werden möglichst viele Gene durch Ansequenzierung der entsprechenden cDNA; die Totalsequenzierung des Genoms wurde begonnen [1-3]. Reis ist relativ einfach transformierbar und Linien mit verschiedenen, gentechnologisch erzeugten Resistenzen (die jährlichen Verluste durch Insekten-, Virus- und Pilzbefall werden auf 45 – 75 Mio. t geschätzt) werden im Freiland getestet. Von besonderer Bedeutung sind Versuche, den Gehalt an Provitamin A im Endosperm des Reiskorns gentechnologisch zu erhöhen [4], um dadurch den in Südostasien noch weit verbreiteten Mangelkrankheiten vorzubeugen.

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**Übersetzungen:**

E rice
F riz
I riso
S arroz

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# Buchweizen



Stand: Januar 2003 > Bearbeitet von: Stefan Gräber
Fachgebiet: Lebensmittelchemie > Unterthema: Getreide und Getreideprodukte, Backwaren

Gelegentlich auch *Heidekorn* genanntes, rötlich-weiß blühendes, 30 –60 cm hohes einjähriges Knöterichgewächs (*Fagopyrum esculentum* Moench u. a. *F.*-Arten, Polygonaceae), das aus der mongol. Steppe stammt u. wegen seiner bucheckerähnlichen Mehlfrüchte in Europa, Asien u. Amerika kultiviert wird.

## Zusammensetzung:

Das B.-Mehl enthält 6,3 % Proteine, 1,1 % Fette, 79,7 % Kohlenhydrate (davon 0,5 % Faserstoffe) u. 12 % Wasser. Der Gehalt an Lysin u. Arginin ist viel höher, der an Cystin niedriger, der an Vitaminen u. Mineralstoffen ähnlich wie bei Getreiden. Der Buchweizen wird zu den Pseudocerealien gezählt.

## Verwendung:

Als Grundstoff für Nahrungsmittel wird Buchweizen als ganze Kerne, Grütze (siehe Graupen), Flocken oder Mehl eingesetzt. Buchweizen kann in Backwaren, als Brei, als Pfannkuchen oder als Teigwaren verspeist werden. Rezepte hierzu sind aus Rußland, Japan, Europa sowie Süd- und Mittelamerika bekannt (*Lit.* [1]). Das Buchweizen-Kraut enthält 1 –5 % Rutin, das technologisch daraus gewonnen werden kann, und wird daher als Tee bei Venenschwäche, Krampfadern und Ödeme angewandt. Bei der Verfütterung an Weidevieh ist wegen des Gehalts an photosensibilisierendem *Fagopyrin* (s. Hypericin) Vorsicht geboten, da dies bes. bei weißbehaartem Vieh zu sog. *Fagopyrismus* (photodynamischer Effekt) führen kann.

## Übersetzungen:

E buckwheat
F blé noir, sarrasin
I grano saraceno, fayopiro
S alforfón, trigo sarraceno

## Literatur:

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# TREATMENT AND CLARIFICATION OF FERMENTED BROTH IN BACTERIAL ENZYME PRODUCTION

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## Summary

Acetic acid, calcium chloride, aluminium sulphate and polyacrylamide were tried for flocculation and settling of suspended solids. Adjustment of pH, centrifugation, precoat filtration with filter aid were studied for clarification of fermentation broth in alpha-amylase production. Combination of aluminium sulphate and polyacrylamide as flocculating agents and precoat filtration was found to be the best strategy for alpha-amylase enzyme recovery from the broth.

## INTRODUCTION

Industrially important alpha-amylase is an extracellular enzyme produced by Bacillus sp. from complex nitrogen and carbon sources. In a typical fermented broth, total solids vary between 50 to 70 g/L, of which enzyme protein (soluble) is at the most 2.5 g/L (Yoneda, 1982). Bacterial fermentation broths are rather difficult to process. Moreover, the broth contains a large amount of colloidal matters which presents serious problem for solid-liquid separation. Both of these factors, together with nature of substrate and bacteria, cause considerable increase in the problems leading to low filtration efficiency and low rate of clarification of broth and poor quality of filtrate containing undesirable colloidal matters. As a result, cost and purity of the final enzyme preparation are adversely affected.

Adding coagulating-flocculating agents help to remove colloidal particles from the broth. The theory of coagulation-flocculation is based on destabilization of colloidal particles. Depending upon the nature of solid particles in the broth and the coagulating agent, particles are destablized through one or more of the following mechanisms: compression of electrical double layer, electrostatic attraction, interparticle bridging, enmeshment. Large stable flocs formed by proper flocculating agents can be removed easily by sedimentation, filtration or centrifugation.

A classical study on the use of various types of flocculating agents for the yeast cell recovery was reported by Gasner and Wang (1970). Use of salts of polyvalent metal ions and synthetic polyelectrolytes have been reported by Volesky and Luong (1985) and Wang (1979). In recent papers, Luhera and Harming (1988) and Hoarse (1988) have also described the treatment of broth with flocculating agents. However, most of these reports except that of Gasner and Wang (1970) did not discuss much about the quantitative techniques for treatment and clarification of bacterial broths.

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In this paper, treatment of broth with various coagulating agents and subsequent clarification of treated broth using different techniques have been discussed. The entire study was focussed on the design of an efficient and cost effective solid-liquid separation process for extracellular bacterial enzyme.

## MATERIALS AND METHODS

**Fermented Broth:** Broths used in this study were obtained by fermentation of complex media of different compositions using Bacillus sp. for a period of 60 - 120 h. Fermentation was conducted in 15L Braun Biostat-E fermenter under identical operating conditions. A typical medium contained (in g/L) corn flour 20, corn steep liquor 25, defatted soya flour 15, maize gluten 10 along with the trace metals and antifoaming agent.

**Pretreatment of Broth:** Fermented broths were treated with various coagulating agents such as glacial acetic acid, calcium chloride at 100 g/L, aluminium sulphhate at 200 g/L, cationic polyacrylamide at 10 g/L individually or in combination with the help of variable speed stirrer fitted with turbine blades.

**Gravity Sedimentation:** Treated broths were allowed to settle in 250 mL graduated cylinder at 10 °C. The height of the clarified zone (distance between upper liquid level and solid liquid interface) was measured in regular intervals of time. The supernatant was subjected to different tests like turbidity, enzyme activity and bacterial count.

**Centrifugation:** Treated broths were centrifuged for 5 - 20 min at 2150 g and the supernatants were subjected to various tests.

**Precoat Vacuum Filtration:** Treated broths were filtered in precoat vacuum leaf filter (area 145 cm under 550 mm Hg vacuum. In all the experiments, initial precoat of 10 mm thickness was prepared using Decamol filter aid. The filter aid of 11 um particle size used in the broth was varied between 0.75 - 1.5% w/v. Filtration rate was estimated by dividing the volume of filtrate by contact time and filtration area.

**Analytical Methods:** Activity of alpha-amylase enzyme was estimated in terms of dextrinizing units (DUN)/mL on the basis of the reduction in intensity of blue colour of the starch iodine complex at 660 nm, showing dextrinization of starch (Bajpai and Bajpai, 1989). Turbidity of the clarified liquid was measured by Nephelometer using hexamine hydrazine sulphate as standard in terms of nephelometric turbidity unit (NTU). Viable count of organism was determined by standard plating technique.

## RESULTS AND DISCUSSION

Efficient and cost effective process of clarification of fermented broth required attention on the following important aspects: (1) minimum loss of enzyme during clarification which results in maximum clarification efficiency, (2) good clarity of filtrate for easy down stream processing (3) presence of any residual coagulating agent in the enzyme and its adverse effect on the final enzyme sample and on the food grade enzyme preparation, (4) minimum operating cost of clarification. The experimental design was made in such a way so as to reach a optimum condition in each of the above four points.

**Effect of Harvesting Time and Medium Composition:** Filtration efficiency, rate and clarity of filtrate reduced with increase in harvesting time as shown in Table 1. All the three values reduced substantially in the broth of 72 h of age. The probable reason could be the excretion of stable matter after 60 h

Table 1. Effect of harvesting time on filtration performance

| Fermentation time (h) | Filtration Efficiency (%) | Filtrate rate ($L/m^2/h$) | Turbidity (NTU) |
|---|---|---|---|
| 48 | 89.7 | 232.5 | 10 |
| 60 | 84.9 | 225.5 | 12 |
| 72 | 76.5 | 190.7 | 26 |

Note.-Glacial acetic acid to pH 5.2 was used as the coagulating aid. The mixing was done on magnetic stirrer for 15 min before filtration.

-Filtration efficiency = percentage of enzyme recovery on filtration

Table 2. Effect of medium composition on clarification

| Type of medium | Turbidity of filtrate (NTU) |
|---|---|
| Complex laboratory medium | 13.5 |
| Cheap commercial medium (dextrin as carbon source) | 55.0 |
| Cheap commercial medium (corn flour as carbon source) | 73.0 |

Table 3. Clarity of centrifugate of the fermented broth after treatment

| Flocculating agents (g/L) | pH | Turbidity (NTU) |
|---|---|---|
| Nil | - | 36.8 x 60 |
| Al 3.0 | 7.0 | 37.5 x 37 |
| Al 3.0 | 5.2 | 28.5 x 37 |
| Al 5.0 | 6.5 | 30.5 x 32 |
| Al 5.0 | 5.2 | 38.7 x 8 |
| Al 7.0 | 5.9 | 35.7 x 4 |
| Al 7.0 | 5.2 | 35.2 x 4 |
| A2 | upto 5.2 | 35.8 x 40 |
| Al 5.0<br>C1 0.1 | 6.5 | 21.0 x 2 |
| Al 5.0<br>C1 0.1 | 5.2 | 27.0 x 2 |
| Al 5.0<br>C1 0.3 | 6.5 | 12.8 |
| Al 5.0<br>C1 0.3 | 5.2 | 11.2 |
| Al 5.0<br>C1 0.5 | 6.5 | 2.4 |
| Al 5.0<br>C1 0.5 | 5.2 | 5.3 |

Al = aluminium sulphate
A2 = acetic acid
C1 = cationic polyacrylamide

or so by Bacillus sp. and/or formation of colloidal matters by the action of undesirable enzyme e.g. protease and unconverted nutrients. These colloidal matters were not flocculated along with other solids by the treatment with coagulating agents. As a result, comparatively less clear filtrate, reduced filtration rate and efficiency were observed. Deterioration in quality of broth was also observed during storage for longer periods even at low temperature of 5°C. Considering all these factors, the harvest time should not be more than 60 h.

While changing the initial medium composition and conducting parallel experiments on treatment of broth and solid-liquid separation, it was observed that the clarification of broth of expensive laboratory medium was the best amongst all the media tried. The clarification performance is given in Table 2 for different media. The presence of more colloidal matters in the filtrate was due to one or more of the following reasons: (1) The solid content in the initial medium was quite high. (2) Organism produced more colloidal matters in the form of insoluble proteins and polysaccharides.

**Effect of Flocculating Agents:** The effect of following coagulating-flocculating systems on the clarification of broth was studied: (a) glacial acetic acid up to pH 5.2 (b) 1 g $CaCl_2$/L and acetic acid to pH 5.2 (c) aluminium sulphate up to 7 g/L (d) aluminium sulphate and acetic acid to pH 5.2 (e) cationic polyacrylamide up to 0.5 g/L and acetic acid to pH 5.2 (f) cationic polyacrylamide, aluminium sulphate and acetic acid (g) cationic polyacrylamide, $CaCl_2$ and acetic acid. While using the systems from (a) to (d), no clear enzyme solution separated out either on sedimentation for 5 h or on centrifugation for 20 min at 2150 g force. However, when cationic polyacrylamide was used either with aluminium sulphate or with aluminium sulphate and acetic acid combination, the clear separation of enzyme solution was observed both on sedimentation and on centrifugation. Clarity of the enzyme solution depended on the concentrations of cationic polyacrylamide and aluminium sulphate. The above phenomenon indicates the synergistic behaviour of two coagulating agents. Similar were the observations with cationic polyacrylamide+$CaCl_2$ in combination with or without acetic acid. But this system was comparatively weak in flocculating properties. Flocculation and subsequent sedimentation was effective at a relatively higher concentration of cationic polyacrylamide of 1 g/L. The clear supernatant enzyme solution was obtained only in the case of aluminium sulphate+cationic polyacrylamide flocculating system. The rate of sedimentation was also much higher in this case. In Table 3, the clarity of the centrifugates of fermented broths treated with different flocculating agents have been given. It is evident from these results that cationic polyacrylamide and aluminium sulphate system does not require any further treatment with acetic acid for pH adjustment.

Performance with various combinations of aluminium sulphate and cationic polyacrylamide and calcium chloride and cationic polyacrylamide is shown in Figure 1. In these cases, the resultant pH was kept at the same level of 5.2 by addition of glacial acetic acid. In sets I to III, clear enzyme solutions were obtained with 1 g cationic polyacrylamide/L along with other electrolytes. However, a decrease in rate of sedimentation was noticed in sets I & III at 1 g polyacrylamide/L. In these cases, colloidal particles were bridged at relatively slower rate. However, with the addition of more aluminium sulphate which helped in the compaction of electrical double layers, interparticle bridging occured at a much faster rate even with smaller dose of cationic polyacrylamide (Set II). Thus higher clarity in supernatant was observed at lower level of polyacrylamide.

Although the best quality of filtrate was obtained by treating the broth with high dose of coagulating agents (5 g aluminium sulphate/L and 1 g polyacrylamide/L) as shown in Fig.2-II but the loss of enzyme was quite high, of the order of 14%. Whereas the loss of enzyme was about 3% when the broth was treated with 0.3 g polyacrylamide/L along with 5 g aluminium sulphate/L. Looking at the enzyme loss and clarity of filtrate/centrifugate, the concentration of polyacrylamide should be less than 0.2 g/L. This would help in minimizing the enzyme loss and treatment cost and reduce the problems in post enzyme processing.

**Effect of Sheer Force and Mixing Time on Clarification:** During the treatment with flocculating agents, broth was subjected to different magnitudes of shear force expressed as impeller tip speed, for varying periods of time. Samples of treated broth were subjected to sedimentation and clarity tests. The results are shown in Fig.2. In case of higher tip speed, 1.44 m/s, no

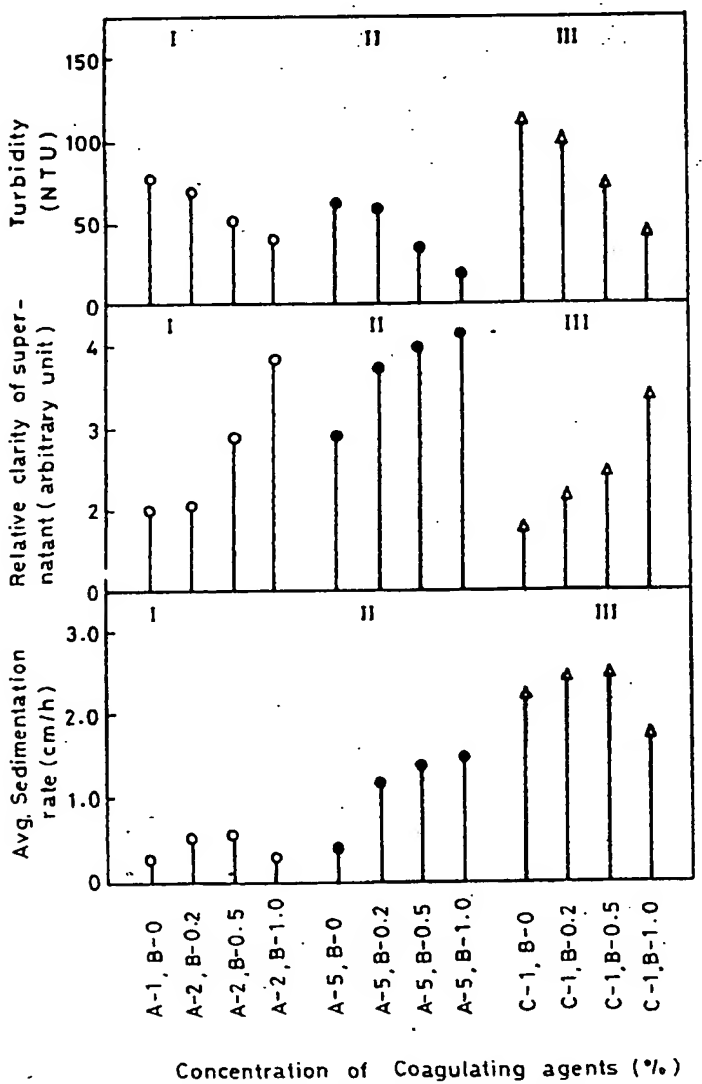


Fig 1. Effect of composition of coagulating agents on clarification of broth. A, B and C refer to aluminium sulphate, cationic polyacryl amide and calcium chloride respectively; I, II and III are sets of experiments.

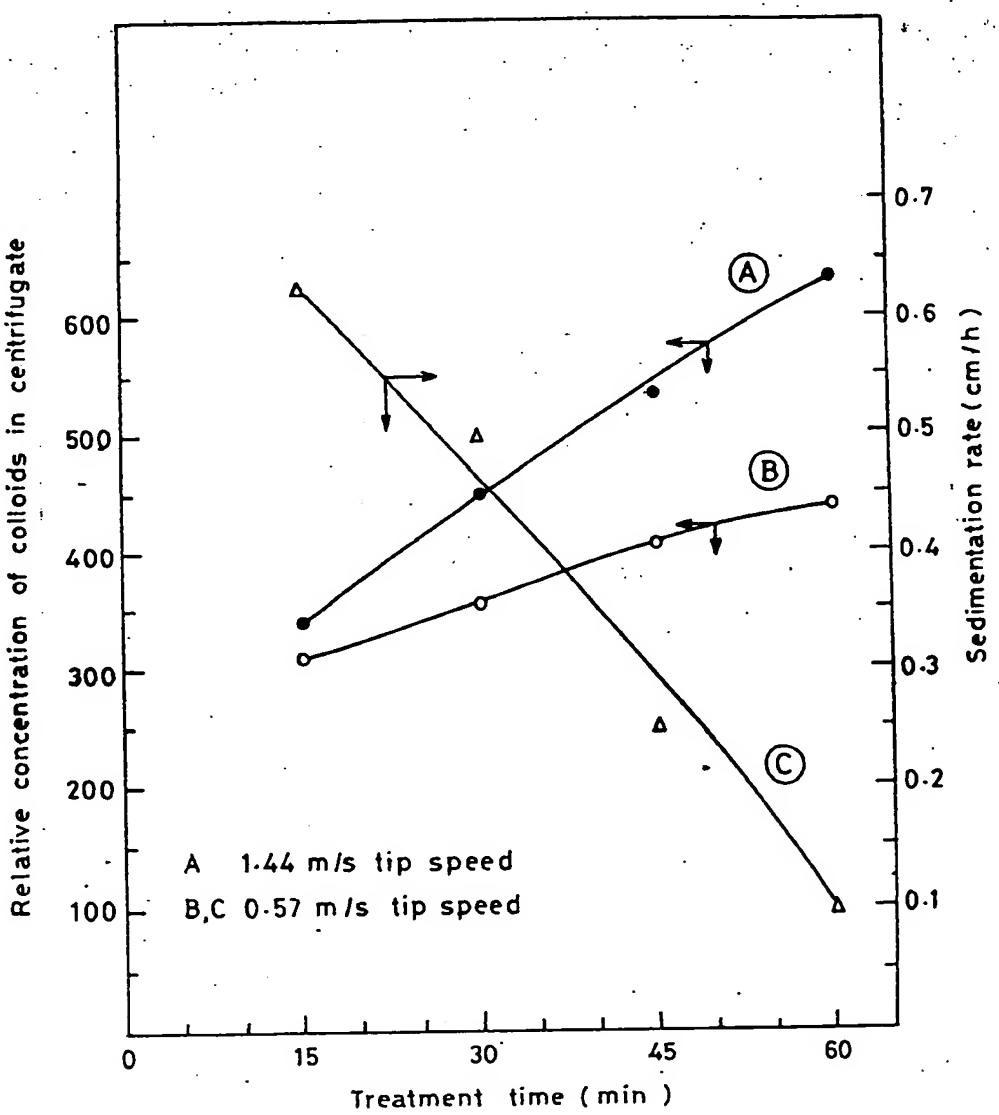


Fig 2. Effect of mixing intensity on clarity and sedimentation rate

clear separation occurred. However, at a low tip speed of 0.57 m/s, clear layer separation was noticed. In addition, the sedimentation rate decreased with the mixing time. This clearly indicates that under high shear and prolong exposure, flocs are disintegrated into smaller particles which prevent settling. Increase in concentration of colloidal matters in centrifugate with tip speed and mixing time confirms the gradual destabilization of flocculated matters.

Before flocs aggregate, microflocs are transporated and attached to each other due to particle collision. The flow conditions in a mechanically agitated flocculating system are turbulent in nature. Under this condition, the flocs are subjected to unequal shear forces. When the local shear stress exceeds the internal binding forces of aggregate, floc break up occurs (Montogomery, 1985). The principle mechanisms of aggregation or break up are related to surface erosion (Argaman and Kaufman, 1970) and floc splitting (Thomas, 1964).

**Selection of Solid-Liquid Separation Process:** The choice was left between centrifugation and precoat vacuum filtration as gravity sedimentation was too slow a process. By the use of 5 g/L aluminium sulphate and 0.3 g/L cationic polyacrylamide as flocculating agent, it was possible to obtain a clear enzyme solution of acceptable quality (turbidity <10 NTU) in 10 min time with the help of moderate centrifugal field whereas it required 15 min when the polyacrylamide dose was reduced to 0.1 g/L. In case of lower dose (0.1 g/L) of polyacrylamide, acceptable quality of filtrate was obtained with an average filtration rate and efficiency of 250 $L/m^2/h$ and 90% respectively. In the clarified filtrate more than 60% reduction in bacterial load was also obtained. This shows that precoat filtration is better than centrifugation. Preliminary economic analysis (not shown) also indicates a saving in processing cost by the use of precoat filtration in place of centrifugation. The high cost of cationic polyacrylamide and centrifugation and the probability of retaining a significant amount of cationic polyacrylamide in the post treatment enzyme solution lead to the selection of precoat vacuum filtration as the suitable process.

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# RECOVERY OF AN ALKALINE PROTEINASE FROM FERMENTATION BROTH USING FLOCCULATION FOR CELL REMOVAL

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**SUMMARY**

Recovery of a bacterial alkaline proteinase after treatment of the fermentation broth with 16 different flocculating additives has been investigated. The polyelectrolyte Sedipur TF 5 was the most effective at 150 ppm and pH 7.0-9.0, giving a 74% yield of enzyme activity.

**INTRODUCTION**

Removal of the cells, solids and colloids from fermentation broth is primary operation step in the recovery of extracellular enzymes (Bautista et al., 1986; Mukhopadhyay et al., 1990) for which vacuum rotary drum filters and continuous disc centrifuges are usually used. A recent development in cross-flow microfiltration has also been applied to enzyme recovery (Whittington, 1990). However, to avoid losses of enzyme activity caused by imperfect clarification, or to prevent the clogging of filters, it is usual to perform some chemical pretreatment of fermentation broth before commencing separation (Aunstrup,1980). The use of flocculating agents to create larger floccs or agglomerates can faciliate and speed-up solid-liquid separation.

We have studied the effect of 16 commercially available organic polyelectrolytes on cell removal and subsequent recovery of extracellular alkaline proteinase.

## MATERIALS AND METHODS

### *Cell growth and enzyme production*

*Bacillus subtilis* CCM 3701 was grown on a complex fermentation medium containing (in g/l): potato starch 60; corn meal 30;casein 10;corn steep liquor 10; fodder yeast 10; diammonium phosphate 1.0. Fermentation was conducted in a 20 l fermenter (L.H.Fermentation, U.K.) with 10 l of working volume at 37°C, 550 rpm, 1.0 vvm air flow, for 48 hours. Alkaline proteinase was produced during stationary phase, starting after 20 hours.

### *Determination of proteolytic activity*

Proteolytic activity (PU/ml) was assayed by the FOLP method (Loginova et al., 1973), using Hammarsten casein (Serva, Heidelberg, FRG) as a substrate.

### *Flocculating additives*

Sixteen samples of flocculating additives were obtained from companies engaged in their commercial production. The additive trade names and the companies which provided these samples are as follows:Praestol 444 K, 555 K, 2530, 3000 (Chemische Fabrik Stockhausen, Krefeld, FRG); Sedipur T 1, TF 5, TF 6 (BASF,Ludwigshafen,FRG); Nalco 4719, 4732, 4762, 4870, 7861, 7862, 7863 (Nalco Chemical Co., Chicago, Ill., USA).

### *Survey of flocculating additives*

In the initial phase 16 commercial flocculants were tested for removal of bacterial cells. Five dosages of flocculant were used corresponding to at least one order magnitude in each direction from the manufacturers recommended dose. Experiments were performed for each additive at room temperature within the pH range of enzyme stability (6.0-10.0).

The flocculation was carried out in 250 ml glass beakers (diameter of 70 mm),mixed with perforated rectangular impeller (40x40 mm). The testing procedure consisted of: (1) pH adjustment of 100 ml of fermentation broth; (2) addition of fresh flocculant solution during 1 minute of intensive mixing (500 rpm); (3) flocculation for 5 minutes at 75 rpm; (4) 30 minute staying without agitation; (5) centrifugation of treated broth (type 310, MPW, Warszawa, Poland) at 4,000 rpm for 10 minutes. The supernatant was used for determination of proteolytic activity and for measurment of optical density at 600 nm in 1 cm cell.

## RESULTS AND DISCUSSION

The efficiency of flocculation process on exocellular proteinase recovery was evaluated using the following three criteria:

(1) high clarification effect. (2) minimal loss of proteolytic activity. (3) low quantity of flocculant.

The results of the screening of different types of flocculating agents are presented in Table 1. Although none of 16 flocculants caused the loss of proteolytic activity, only two mild anionic polyelectrolytes (Sedipur T 1 and Sedipur TF 5) showed satisfactory clarification effect. This fact is in accordance with the conclusions of Gasner and Wang (1970) which found that, despite of the negatively charged cell surface, anionic polyelectrolytes can be used as flocculants. This effect is accounted for by an excess of multivalent cations in the fermentation broth which forms an adsorbed layer on the cell surface changing its flocculation characteristics.

In the next experiment, the clarification efficiency of Sedipur TF 5 was verified in a larger volume. The fermentation medium was separated after treatment with flocculant on the disc centrifuge. As can be seen from the Table 2, total yield of proteolytic activity after centrifugation and washing the sediment with water was only 74% as compared with 98% value achieved in the batch centrifuge. Considerable amount of proteolytic activity remained in the slurry. To overcome this problem it would be necessary to increase the quantity of washing water,but this approach seems to be disadvantageous from the economic point of view. The other way is looking for new types of flocculating agents which form larger and more stable agglomerates.

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TABLE 1. Effect of different flocculants on the clarification of fermentation broth

| Flocculant | Ionogenity | Clarification degree |
|---|---|---|
| Praestol 444 K | wc | – |
| Praestol 511 K | wc | – |
| Praestol 550 K | mc | – |
| Praestol 555 K | sc | – |
| Praestol 2530 | ma | – |
| Praestol 3000 | n | – |
| Sedipur T 1 | ma | + |
| Sedipur TF 5 | ma | ++ |
| Sedipur TF 6 | ma | – |
| Nalco 4719 | wc | – |
| Nalco 4732 | mc | – |
| Nalco 4762 | sc | – |
| Nalco 4870 | sc | – |
| Nalco 7861 | n | – |
| Nalco 7862 | wa | – |
| Nalco 7863 | ma | – |

Flocculating conditions : Fermentation broth (10,000 PU/ml, pH 7.0, 20°C) was treated with 150 ppm of flocculants. The degree of clarification was evaluated after centrifugation, according to absorbance in supernatant at 600 nm as good (++) if $A < 0.5$, satisfactory (+) if $A > 0.5$ and unsufficient (–) if the sample remained opaque. The ionogenity of flocculants is expressed as weak anionic (wa), mild anionic (ma), strong anionic (sa), nonionic (n), weak cationic (wc), mild cationic (mc) and strong cationic (sc).

TABLE 2. Recovery of alkaline proteinase after clarification of fermentation broth treated with Sedipur TF 5

| medium | proteolytic activity (PU/ml) | volume (ml) | total proteolytic activity ($PUx10^6$) | yield of enzyme (%) |
|---|---|---|---|---|
| fermentation broth | 11,600 | 1,000 | 11.6 | 100 |
| supernatant | 8,942 | 960 | 8.6 | 74 |

Operation conditions : Fermentation broth (pH 9.0, 20°C) was treated with Sedipur TF 5 (150 ppm) and separated on disc centrifuge (Gyrotest, α– Laval, Sweden) at 10,000 rpm. Proteolytic activity and the yield of alkaline proteinase were determined after connection of supernatant with 250 ml of washing water.